Ho-Yi Tang
University of Oxford

Mitochondrial transfer – the future for inherited mitochondrial disease?

Mitochondria are tiny structures, or organelles, in our cells which provide the energy for our cells to function. Distinctly separate from the nucleus in a cell, they contain their own DNA and have their origins in the endosymbiotic merging of our unicellular ancestors with free-living bacteria. As such a vital part of our cell's machinery, it is hardly surprising that devastating disease can result when our mitochondria do not function effectively.

An estimated 1 in every 4000-5000 children born each year shows signs of serious mitochondrial dysfunction [1]. In the most severe cases, children do not live beyond infancy or childhood, or are affected by debilitating health problems. A diagnosis of mitochondrial disease currently offers no cure, and only limited treatment [2, 3]. This essay explores the unique challenges of mitochondrial DNA-related disease, and considers emerging techniques which might offer hope to parents at risk of passing on mitochondrial disease to their offspring. These pioneering mitochondrial replacement therapies (MRTs) aim to circumvent future cases of mitochondrial disease entirely by incorporating mitochondria and their genetic material from an unrelated donor. In the public domain, MRTs have had vocal detractors, and have undeservedly, if understandably, gained an association with the 'slippery slope' towards unethical 'designer babies'. However, through considered public consultation and strong support from respected scientific bodies, MRTs look set to become the next clinical intervention available to avoid devastating disease and to offer hope to at-risk parents.

Mitochondrial disease: Genetic disease like any other?

Mitochondria have their own distinct genomes containing 37 genes which code for crucial elements of mitochondrial function, with a multitude of additional genes located on nuclear DNA. As a result, defects in mitochondrial function can stem from either mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) [4]. Such defects in mtDNA have been recognised since 1988, with the identification of a point mutation in mtDNA as the root of Leber's hereditary optic neuropathy [5, 6].

Where the defect is present in mtDNA, the situation is somewhat more complicated than in nDNA, where an individual inherits genes from both the mother and father. Instead, each oocyte receives multiple mitochondria from the mother alone, resulting in multiple copies of mtDNA being present. Numbering in their thousands, these are most often genetically identical (termed homoplasmy) but sometimes are not (heteroplasmy). The earliest conclusive evidence for mtDNA heteroplasmy in humans came from Holt, Harding and Morgan-Hughes in 1988 [7]. By investigating whether the observed maternal inheritance of mitochondrial myopathies was due to an mtDNA mutation, the authors discovered that in muscle biopsies from patients, a deletion only present in a

fraction of mtDNA copies resulted in two populations of differently sized mtDNA, compared to a control showing just one population. Heteroplasmy has since proved the cornerstone on which much of our understanding of mitochondrial inheritance and disease has been deduced.

Although the precise mechanisms of mitochondrial inheritance, typically investigated using mouse development models, are still a source of contention [8, 9], it is evident that there is huge variation in mtDNA inheritance due to a segregation bottleneck, which is observable in cases of heteroplasmy [10]. Simply put, if a mother has any number of mutated mtDNA sequences in among healthy ones, the share of mutant or healthy mtDNA her offspring receive is unpredictable. As a result, a mother with a low level of mutated mtDNA may be asymptomatic or have mild disease, but their child may be more severely affected as they inherit a disproportionately high level of mutated mtDNA [11].

The resulting implications for mitochondrial disease are not only an exclusively maternal pattern of inheritance but also a high degree of unpredictability over the extent to which pathological conditions are transmitted. This variation in levels of mutated mtDNA compared to normal DNA, together with the huge range of clinical presentations that results from mitochondrial disease affecting any organ system in the body, therefore make mtDNA-related disease a beast of a very different nature compared to the larger body of nDNA-related disease, especially when considering diagnostic tests or targeted gene therapies.

Why MRTs?

The significance of MRTs lies in their potential as a way to prevent transmission of mitochondrial conditions from mother to child entirely, whilst ensuring the child is genetically related to the mother. Researchers headed by Professor Doug Turnbull at the University of Newcastle have recently demonstrated successful proof of principle experiments in abnormally fertilised human embryos, by removing the pronucleus, containing nuclear DNA, and transferring it to a donor embryo which had had its own pronucleus removed [12]. The embryo produced from this pronuclear transfer (PNT) contained DNA from three sources: the two parents providing the nuclear genome, and a third individual whose mitochondrial DNA was then present in the embryo. A variant of this technique, where the maternal spindle of nuclear DNA is transferred to a donor oocyte prior to fertilisation (maternal spindle transfer, or MST), has been successfully employed in rhesus monkey gametes by Tachibana *et al.* [13] with no adverse effects reported when the offspring were born at term.

While PNT and MST appear to be viable from a technical viewpoint and have been deemed ‘not unsafe’ on current evidence by the Human Fertilisation and Embryology Authority (HFEA),

proposals for its use have been criticised on many grounds. Some safety concerns centre on the fact that mitochondrial-nuclear interactions are still poorly understood [14], and that some of these, including the possibility of endosymbiotic gene transfer between maternal and donor genetic material, could be harmful. There are also concerns over the ethics of germline manipulation, as any changes to mitochondrial genomes can then be inherited by subsequent generations, and the fact that PNT involves the destruction of embryos, an ethical barrier for some.

Under public scrutiny: does MRT deserve its 'three-parent baby' moniker?

Media attention has mainly focused on two sensationalised aspects of MRT. Firstly, the concept of a 'three parent baby' has captured the imagination of journalists and hints at a perceived threat to traditional family and societal structures. Interestingly, any children born as a result of MRT would not be the first to have a genetic connection to three separate people – cytoplasmic transfer, another related technique, was successfully employed during in vitro fertilisation (IVF) as early as 1997 in the United States with no observable ill effects [15, 16].

To address the concerns about the status of 'genetic' parents being undermined, the Nuffield Council for Bioethics in their 2012 report on the ethics of MRT took great pains to address the concepts of 'identity' and 'parenthood' in a societal context as well as a genetic one [17]. The report concluded that it was 'not legally or biologically accurate to refer to the mitochondrial donor as a mother or 'third parent' of the resulting child' [18]. Similarly, advocates for MRT such as Professor Julian Savulescu and Lord Robert Winston have highlighted MRT's similarity to more publically accepted procedures such as organ or bone marrow transplants, stressing that such interventions do not usually change our perception of an individual's identity [19, 20].

In addition, a 'slippery slope' argument has been employed by critics, arguing that MRT is simply the thin end of a wedge that will ultimately allow parents to select 'desirable' characteristics, thus resulting in the creation of 'designer babies' [21]. mtDNA has no involvement in determining conventionally 'desired' characteristics in a child, and the proposed legislation for licensing MRT makes a clear distinction between the mitochondrial and nuclear genomes. Since it is clear, then, that nuclear genome modification will not be permitted, the blanket labelling of MRT as 'designer genetic modification' is unreasonable as it runs the risk of shutting down debate over a procedure with enormous potential to improve lives.

What can MRT offer over existing measures?

Current options for families affected by mitochondrial disease are scarce, highlighting the clear need for interventions such as MRTs. Existing genetic counselling includes prenatal diagnostic tests such as chorionic villus testing, used to inform pregnant women about the chances of their child being affected. Since 2006, preimplantation genetic diagnosis (PGD) has allowed for a more active or interventional approach to pre-birth diagnosis. By removing one or two cells from the early embryo and screening them for specific diseases, it is possible to select the embryos shown to be at low risk of being affected by the condition for use in the rest of the IVF process [14].

However, the fact that mutated mtDNA can make up just a proportion of total copies of mtDNA in the cell, and still cause disease, has led to concerns that prenatal testing and PGD are far from reliable tests in cases of mitochondrial disease [14]. A further option for couples wishing to have a child unaffected by mitochondrial disease is oocyte donation, theoretically ensuring that no disease-causing mtDNA is passed to the child. This may be of particular value to women with a high proportion of mutated mtDNA, whose children would be at significant risk of severe disease and for whom PGD would not be likely to be useful, but this has its own disadvantages: donor oocytes are consistently in short supply, and the child would have no genetic link to its mother.

While MRT bypasses many of the above issues, by providing a much higher degree of certainty of low mtDNA carryover from the mother to the child, major limitations still remain. It will be of no assistance in sporadic cases, only those where couples know of their risk and receive genetic counselling. Other issues remain: the limited oocyte supply, and the ethical challenges regarding germline modification and embryo destruction. MRT also cannot be applied in cases where the mitochondrial defect stems from a mutation in nDNA rather than mtDNA, so it is by no means a magic bullet for all types of mitochondrial disease.

Conclusions

The 35 years since the first success of *in vitro* fertilisation has seen over 5 million babies born using assisted reproductive techniques (ARTs). Its impact has not only been on treating infertility, but more recently in the use of preimplantation genetic diagnosis to prevent often devastating inheritance of genetic conditions. The next chapter of ARTs in clinical practice could soon be underway. Pending further safety tests, the passing of The Human Fertilisation and Embryology (Mitochondrial Donation) Regulations 2015 in Parliament in February this year paves the way for MRT to be introduced into clinical use in the near future. Under the regulation of the HFEA, this would make the UK the first country in the world to offer MRTs to affected families. This ongoing drive towards the introduction of MRT is based on the general consensus that, where there exists such a technology that offers the

chance for children to be born free of devastating disease, there is an ethical obligation to work towards making this a reality in the safest possible manner.

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Details of submission

I am entering the undergraduate competition. I am in my 4th year of studying at the University of Oxford, reading for a BM BCh in Clinical Medicine (undergraduate entry course).

Tutor:
Dr Tim Littlewood
C/O Oxford University Medical School Office
Academic Centre level 2
John Radcliffe Hospital
Oxford OX3 9DU
tim.littlewood@ouh.nhs.uk

Correspondence:
Ms Ho-Yi Tang
Merton College
Merton St
Oxford OX1 4JD
ho-yi.tang@merton.ox.ac.uk